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I, LEANNE MYNOTT, MANAGER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. 2004904367 for a patent by VITAL HEALTH SCIENCES PTY LTD as filed on 03 August 2004.

WITNESS my hand this
Seventeenth day of March 2005

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Patents Act 1990

PROVISIONAL SPECIFICATION

Invention title: ALKALOID FORMULATIONS

The invention is described in the following statement:

Alkaloid Formulations

Field of the invention

The present invention is directed to formulations comprising alkaloids. More specifically but not exclusively it relates to formulations comprising alkaloids and phosphate derivatives of electron transfer agents.

Background of the invention

In this specification, where a document, act or item of knowledge is referred to or discussed, this reference or discussion is not an admission that the document, act or item of knowledge or any combination thereof was at the priority date: part of common general knowledge, or known to be relevant to an attempt to solve any problem with which this specification is concerned.

Alkaloids

There is a long history of the use of alkaloids for medicine. These compounds were originally extracted from plants and include nitrogenous compounds having physiological actions on humans as drugs and poisons. The term "alkaloids" as used in this specification includes all natural and synthetic active compounds containing primary, secondary or tertiary amine substituents. The amine may be incorporated into one or two rings, but non-cyclic structures are also included. For example, this includes:-

- tertiary amines which:-
    - are alicyclic with the nitrogen atom as a common member of three rings (eg. Morphine,. Atropine, Quinine); or
    - are cyclic where the nitrogen is incorporated into a single ring and alkylated (eg. Nicotine, Fenspiride); or
    - have no cyclic structure incorporating the nitrogen (eg. Flurazepan);
- secondary amines where the nitrogen is incorporated into an alicyclic structure (eg Conline, Fendiline) or a linear structure (eg. Epinephrine);
- primary amines (eg. Ephidrine);
- pyridines (eg Nicotine);
- methamidine derivatives;
- quinolines (eg. Cinchonine); and
- guanidines (eg. Arginine).

Most alkaloids are not water soluble but are soluble in organic solvents. However, all alkaloids are basic and will combine with acids to form crystalline salts which are usually at

least partially water soluble. Typically, alkaloids are administered as salts either orally or by intravenous injection. The alkaloids are a class of drugs that are not commonly administered transdermally because the hydrophilic nature of the salts usually limits transdermal transport. Morphine and atropine are examples of clinically useful alkaloids that are not administered transdermally. Further, it is desirable to improve oral delivery of alkaloids since some of them are thought to act through the lymphatic system.

Topical administration

Topical administration refers to the application of a drug directly to a part of the body and includes transdermal administration (application to the skin) and buccal administration (application to the inside of the mouth).

The skin is the largest organ of the body and functions to protect the internal organs from external chemical, physical and pathological hazards. Normal skin is divided into three layers: the epidermis, the dermis, and subcutaneous tissue. The outer cornified layer of the epidermis, the stratum corneum, possesses properties of strength, flexibility, high electrical impedance and dryness that retards penetration and proliferation of micro-organisms. The stratum corneum is also the principle barrier to transdermal drug absorption.

The art of transdermal delivery includes the application of drugs in the pure state or as formulations which typically include substances that enhance the rate of transport through the skin. Historically transdermal delivery was as ointments, creams, poultices and plasters to give effective contact with the skin. More recently, the technology has been improved by making the plaster into a "patch" which has better adhesion to the skin and improved control over the rate of transport.

Transdermal delivery has been recognized to offer several potential benefits including achieving blood levels similar to those achieved by slow intravenous infusion but without the inconvenience; better control of absorption and metabolism compared to oral administration; continuity of drug effect especially of drugs with short half lives; equivalent efficacy with reduced drug dosage due to by-pass of hepatic first pass elimination; lower risk of under or overdosing; and better patient compliance through simplification of a dosage regime.

Not every drug can be administered transdermally at a rate sufficiently high enough to achieve blood levels that are therapeutically beneficial for systemic medication. Drugs with similar molecular weights and sizes for example may absorb across the skin at different rates. Skin enhancers and various formulation techniques have been developed to improve drug absorption through the skin. But concern has been raised with respect to long term risk because increased drug permeability is achieved at the cost of damaging a fundamentally important protective layer of the skin.

Current strategies to improve transdermal therapy have not been universally successful and there is scope for further improvement. In particular, there is a need for use of transdermal delivery systems capable of delivering alkaloids.

There has also been increased interest in buccal delivery since this method of delivery avoids metabolism by the liver which can be a problem when drugs are administered orally. Typically, the drug is formulated in a lozenge which is placed under the tongue. The lining of the mouth does not have an equivalent of the stratum corneum on the skin so it is not as difficult to administer drugs by buccal delivery, but this method of administration is not commonly used because the rate of transport may be low, achieving an ineffective result if the buccal membranes do not allow permeation or active transport.

Efforts have been made in the past to improve the topical administration of drugs. For example, international patent application no PCT/AU03/00998 discloses a carrier for pharmaceuticals wherein the carrier comprises a complex of a phosphate derivative of a pharmaceutically acceptable compound, for example, laurylaminodipropionic acid tocopheryl phosphates. PCT/AU03/00998 discloses that the tocopheryl phosphate is complexed to a complexing agent selected from the group consisting of amphoteric surfactants, cationic surfactants, amino acids having nitrogen functional groups and proteins rich in these amino acids. This carrier has been shown to improve the topical administration of testosterone, estrogen, atropine and morphine. However, in relation to morphine and atropine, further improvement in skin penetration was desired.

Oral administration

Many drugs are administered orally, but a large number of potentially useful drugs are rejected because they are unable to pass through the intestinal walls. It is understood that substances such as fats are efficiently transported through the intestines, but many others such as tocopherol are poorly transported. There is thus a need for systems which enable improved oral administration of alkaloids.

Summary of the invention

It has been found that there is a significant improvement in administration when an alkaloid compound is complexed directly to a phosphate derivative of an electron transfer agent. For example, the administration of morphine was improved when it was complexed directly to tocopheryl phosphate.

According to the present invention, there is provided an alkaloid formulation comprising one or more complexes formed by reacting one or more alkaloids with one or more phosphate derivatives of one or more electron transfer agents.

Preferably, the phosphate derivative of a electron transfer agent is selected from the group comprising one or more phosphate derivatives of tocopherol.

Preferably, the alkaloid formulation is administered topically or orally.

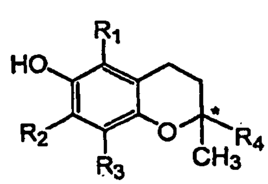
According to a second aspect of the invention, there is provided a method for improving the efficacy of alkaloids, said method comprising the step of complexing the alkaloid with one or more phosphate derivative of one or more electron transfer agents.

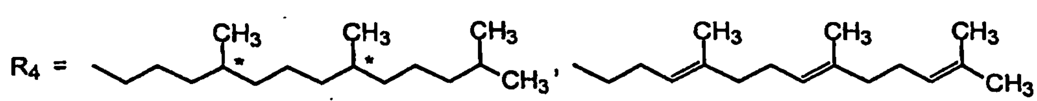
The present invention also provides for the use of one or more complexes formed by reacting one or more alkaloids with one or more phosphate derivatives of one or more electron transfer agents, together with excipients in the manufacture of a formulation.

The present invention also provides a pharmaceutical composition comprising one or more complexes formed by reacting one or more alkaloids with one or more phosphate derivatives of one or more electron transfer agents, such as phosphate derivatives of tocopherol.

The term "electron transfer agents" is used herein to refer to the class of chemicals which may be phosphorylated and which (in the non-phosphorylated form) can accept an electron to generate a relatively stable molecular radical or accept two electrons to allow the compound to participate in a reversible redox system. Examples of classes of electron transfer agent compounds that may be phosphorylated include hydroxy chromans including alpha, beta, gamma and delta tocols in enantiomeric and racemic forms; quinols being the reduced forms of electron transfer agent K1 and ubiquinone; hydroxy carotenoids including retinol; calciferol and ascorbic acid. Preferably, the electron transfer agent is selected from the group consisting of tocopherol and other tocols, retinol, electron transfer agent K1 and mixtures thereof.

More preferably, the electron transfer agent is selected from the group consisting of the tocols and mixtures thereof. The tocols include all isomers of derivatives of 6:hydoxy 2:methyl chroman (see structure below) where R_1, R_2 and R_3 may be hydrogen or methyl groups, that is, the α-5:7:8 tri-methyl; β-5:8 di-methyl; γ-7:8 di-methyl; and δ 8 methyl derivatives. In the tocopherols, R_4 is substituted by 4:8:12 tri-methyl tridecane and includes various stereoisomers and optical isomers (chiral centres are indicted by the *). In the tocotrienols, R_4 is substituted by 4:8:12 tri-methyl trideca-3:7:11 triene and the 2 position may be stereoactive as R or S stereoisomers. Most preferably, the electron transfer agent is α-tocopherol.





The term "phosphate derivatives" is used herein to refer to compounds covalently bound by means of an oxygen to the phosphorus atom of a phosphate group thus forming a carbon –

oxygen –phosphorous bond. The oxygen atom is typically derived from a hydroxyl group on the electron transfer agent. The term includes the acid forms of phosphorylated electron transfer agents, salts of the phosphates including metal salts such as sodium, magnesium, potassium and calcium and any other derivative where the phosphate proton is replaced by other substituents such as ethyl or methyl groups or phosphatidyl groups. The term includes mixtures of phosphate derivatives, especially those which result from phosphorylation reactions, as well as each of the phosphate derivatives alone. For example, the term includes a mixture of mono-tocopheryl phosphate (TP) and di-tocopheryl phosphate (T2P) as well as each of TP and T2P alone. Suitable mixtures are described in international patent application no PCT/AU01/01475.

Preferably, the one or more phosphate derivatives of one or more electron transfer agents is selected from the group consisting of mono-tocopheryl phosphate, di-tocopheryl phosphate and mixtures thereof. Most preferably, the one or more phosphate derivatives of one or more electron transfer agents is a mixture of mono-tocopheryl phosphate and di-tocopheryl phosphate.

In some situations, it may be necessary to use a phosphate derivative such as a phosphatide where additional properties such as increased water solubility are preferred. Phosphatidyl derivatives are amino alkyl derivatives of organic phosphates. These derivatives may be prepared from amines having a structure of $R_1R_2N(CH_2)_nOH$ wherein n is an integer between 1 and 6 and R_1 and R_2 may be either H or short alkyl chains with 3 or less carbons. R_1 and R_2 may be the same or different. The phosphatidyl derivatives are prepared by displacing the hydroxyl proton of the electron transfer agent with a phosphate entity that is then reacted with an amine, such as ethanolamine or N,N' dimethylethanolamine, to generate the phosphatidyl derivative of the electron transfer agent. One method of preparation of the phosphatidyl derivatives uses a basic solvent such as pyridine or triethylamine with phosphorous oxychloride to prepare the intermediate which is then reacted with the hydroxy group of the amine to produce the corresponding phosphatidyl derivative, such as P cholyl P tocopheryl dihydrogen phosphate.

The alkaloid formulation may be administered to humans or animals through a variety of dose forms such as supplements, enteral feeds, parenteral dose forms, suppositories, nasal delivery forms, dermal delivery including patches and creams, buccal delivery forms. Oral or buccal delivery may specifically suit alkaloids which have low water solubility.

Preferably, oral alkaloid formulations according to the invention further comprise an enteric coating. The enteric coating protects the complexes from the acidic environment in the stomach. Oral formulations may take the form of tablets, powders, chewable tablets, capsules, oral suspensions, suspensions, emulsions or fluids, children's formulations, enteral feeds, nutraceuticals, and functional foods.

The dose form may further include any additives routinely used in preparation of that dose form such as starch or polymeric binders, sweeteners, coloring agents, emulsifiers, coatings and the like. Other suitable additives will be readily apparent to those skilled in the art.

Brief Description of the Drawings

Figure 1: Effect of various atropine formulations on heart rate in pigs.
Figure 2: Typical differential of heart rate versus time curve.
Figure 3: Effect of various base creams on heart rate in pigs.
Figure 4: Typical heart rate versus time curve.
Figure 5: Effect of treatment and time flinch response after heat probe application
Figure 6: Effect of morphine sulphate 5 mg/kg, morphine with TPm 5 mg/kg and control on paw withdrawal latency in rats, tested over 3 hours (pooled data).

Examples

The following examples are illustrative of the present invention.

Example 1

This example investigates the transdermal delivery to pigs of atropine in a formulation according to the invention. This experiment investigated the effects of dermal penetration of atropine when applied in gel form on heart rate of pigs.

Methods and materials

Atropine (20 mg/kg) was formulated in the following base creams for testing. In addition to the components specified below, all of the creams contained the following: 12% Ultrez-10 Carbomer-3% solution, 0.25% Triethanolamine, 0.1% Surcide DMDMH and Deionized Water up to 100%

Code	Composition
A	1.27% Deriphat 160
B	7.5% of 40% disodium lauryliminodipropionate monotocopheryl phosphate and lauryliminodipropionate ditocopheryl phosphate
C	0.77% Arginine
D	7.5% of 40% arginine monotocopheryl phosphate and arginine ditocopheryl phosphate
E	7.5% of 40% arginine mono tocopheryl phosphate
F	3% mono tocopheryl phosphate
G	3% mono tocopheryl phosphate and ditocopheryl phosphate
H	7.5% disodium lauryliminodipropionate mono tocopheryl phosphate

Code	Composition
I	1.5% Triethanolamine
J	Tocopheryl phosphate and di-tocopheryl phosphate

Ten male crossbred (Large white x Landrace) pigs (initial average weight 51.5 kg and final average weight of 61.0 kg) were utilised in this experiment. Four days prior to the study fourteen pigs were weighed and randomly allocated to individual pens (1.75 m x 0.65 m) in the experimental facility for an acclimatisation period. During this period the hair on the back of the pigs was removed with animal clippers (Oster – U.S.A) followed by regular shaving with an electric human shaver (Philishave HQ5041 – Philips Aust Pty Ltd). Elastic belts were also placed around the chest of the pigs to accustom them to wearing the heart rate monitors. At the start of the experiment the ten pigs that adapted best to the environment and regular handling were selected and housed such that there were no pigs in adjacent pens. This physical separation of the pigs avoided any potential conflict between signals from the heart rate monitors which all operated at the same frequency. The ten pigs were divided into two groups of five (odd and even numbers) and utilised on alternate days in the experiment. An experimental replicate was therefore performed over two consecutive treatment days. Within each replicate the ten pigs were randomly assigned to one of the ten treatment groups, therefore each pig was used for data capture on five occasions, and each treatment was applied five times.

On each measurement day by about 08:00 the five pigs under experiment were weighed, fitted with heart rate monitors and recording of heart rate at 1-minute intervals commenced. Human heart rate monitors (Polar Sport Tester PE4000 - Polar Electro Finland) were used to capture heart rate data. Chest belts with in-built sensors and transmitters were fitted around the pig's chest just behind the front legs. These belts had a liberal coating of an ultra-sonic gel (Virbac Aust Pty Ltd) applied to the sensor contact areas to ensure a good heart rate signal was obtained. A second belt fabricated from 100 mm wide elastic and velcro was placed around the pigs over the transmitter belt. This belt protected the transmitter from physical damage and included a pocket for storage of the monitor recording unit (similar to a wristwatch) during the recording period. An area on the back of the pigs was then shaved with the electric human shaver. Within this shaved area a template and permanent marker was used to outline a rectangular treatment application area of 172.5 cm^2 (75 x 230 mm). Feed was then offered at 100 g/kg liveweight $^{0.75}$ (eg: 55 kg pig = 2020 g/d). Treatment application was begun at least 1 h after the commencement of heart rate recording. Three staff wearing protective rubber gloves applied each of the test formulations in 5 ml syringes. This involved rubbing the products into the skin of the pig while an assistant directed warm air from an electric hair dryer onto the treatment area. Rubbing was discontinued after

approximately 8 to 10 minutes when the skin surface became tacky to touch. Three (10 x 12 cm) transparent dressings (Tegaderm – 3M Health Care U.S.A.) were then applied over the treatment area. Following treatment application the pigs were left undisturbed for the remaining 6 to 7 hours of the recording period. Syringes and gloves used in treatment applications were weighed before and after application to enable accurate calculation of the actual doses applied to the pigs. At the conclusion of the recording period, the heart rate monitors and the transparent dressings were removed and the treatment application area was washed down with warm water containing a small quantity of a liquid handwash.

Results

The average heart rate responses (corrected for baseline using pre-treatment values as a covariate) to the various atropine preparations are presented in Figure 1. All preparations appeared to elicit a response although there was considerable variation both within and between preparations. As anticipated there was no effect of preparation on basal heart rate, which averaged approximately 150 bpm (Figure 1 and Table 1). In most pigs there was a transient increase in heart rate around application, which probably was related to animal excitement during handling. In some pigs this declined with time suggesting some acclimatisation to sample application. Fortunately, including replicate and pig in the statistical model could at least partly account for any acclimatisation. After 10-15 minutes heart rate generally commenced to increase, before reaching a brief peak or plateau and then declining (Figure 2). However, there were significant differences in average heart rates over at least the first 2 hours after application. For example, over the first hour after treatment the average heart rates of pigs receiving Treatments C and G (176 and 180 bpm, respectively) were significantly higher than that of pigs receiving Treatments B, D I and J (155, 155, 155 and 154 bpm, respectively) with the remaining treatments being intermediate (Table 1). Over the second hour after treatment the average heart rates of pigs receiving Treatments G and H (196 and 190 bpm, respectively) were significantly higher than that of pigs receiving Treatments B, D, E, I and J (170, 169, 170, 164 and 165 bpm, respectively) with the remaining treatments being intermediate. The responses were not as pronounced over the third hour post treatment although pigs receiving Treatments G and H (168 and 171 bpm, respectively) had higher heart rates than pig receiving Treatments E and I (148 and 144 bpm, respectively). Beyond 3 hours the differences between treatments were no longer evident. Differences from baseline reflected the mean heart rate data with Treatments A, C, G and H providing the greatest increases in heart rate over the first 2-3 hours after treatment (Table 2).

Peak heart rate was significantly higher in pigs receiving Treatments A and G (219 and 224 bpm, respectively) than in Pigs receiving Treatments D and J (195 and 194 bpm, respectively) (Table 2). There was no significant difference in the time taken to reach peak

heart rate, which was achieved approximately 60 minutes after application of the treatments (Table 1 and Figure 1). After the transient increase in heart rate after treatment application and a delay time of approximately 10-20 minutes, there was an apparent increase in heart rate in all treatment groups. The slope of the increase in heart rate after treatment was greatest in pigs receiving Treatment G (2.7 bpm/min) than in pigs receiving Treatments B, D and E (1.0, 0.9 and 1.2 bpm/min). After peaking or reaching a plateau, heart rate then generally gradually declined. The slope of the decrease in heart rate was significantly lower in pigs receiving Treatments F, G and H (0.40, 0.42 and 0.37 bpm/min, respectively) than in pigs receiving Treatments D and J (0.75 and 0.89 bpm/min, respectively) with the remaining treatments being intermediate. As another indicator of the ability to reach and then maintain an elevation in heart rate the data were also expressed as a ratio of the two slopes. The magnitude of this ratio was significantly greater in pigs receiving Treatments H and G (6.4 and 4.8, respectively) than in pigs receiving Treatments D and J (1.2 and 1.6, respectively) with the rest intermediate.

As anticipated there was no effect of base cream preparation on basal heart rate, which averaged approximately 140 bpm (Figure 3 and Table 3). In contrast to the atropine treatments, in most pigs there was not a transient increase in heart rate around application, which probably was related to a reduction in animal excitement during handling over the course of this study. Since baseline heart rate was also somewhat lower than the average during the atropine treatment phase (140 vs 150 bpm) this suggests some acclimatisation. Because of this it would have been more desirable to include Base creams and Atropine treatments together so that true effects of the application procedure could be determined. Regardless, there did not appear to be any major effects of Base creams on heart rate over at least the first 2 hours of treatment (Figure 3 and Table 3). During the third hour the average heart rate in pigs receiving Treatment E (135 bpm) were significantly greater than in pigs receiving Treatments F and I (119 bpm) but the biological importance of this is unknown. When expressed as difference from baseline these differences disappear. A typical heart rate versus time curve is given in Figure 4.

Table 1. Effect of various atropine preparations on average heart rate over 60 minute intervals.

	A	B	C	D	E	F	G	H	I	J	sed	χ^2
Heart rate (bpm)												
-60 – 0 min	148	147	148	154	148	152	151	149	150	146	5.52	0.916
0 – 60 min	173	155	176	155	165	162	180	170	155	154	9.33	0.007
60 – 120 min	186	170	184	169	170	175	196	190	164	165	10.91	0.011
120 - 180 min	161	156	162	154	148	165	168	171	144	153	10.46	0.124
180 – 240 min	145	148	146	149	139	156	152	164	144	149	9.87	0.353
240 – 300 min	144	146	150	142	147	147	146	155	139	136	7.93	0.471
300 – 360 min	143	142	144	131	137	142	147	150	135	136	7.60	0.271
Difference from baseline (bpm)												
0 – 60 min	24.2	8.7	28.5	1.1	16.1	10.3	30.7	20.0	4.9	7.1	10.41	0.021
60 – 120 min	37.8	22.8	35.6	14.1	21.7	22.6	46.8	40.9	13.2	19.0	12.58	0.045
120 - 180 min	13.0	8.9	13.2	-1.4	-0.8	12.8	20.1	21.2	-6.8	8.0	11.71	0.196

Table 2. Effect of various atropine preparations on average heart rate over 60 minute intervals.

	A	B	C	D	E	F	G	H	I	J	sed	χ^2
Log peak rate (bpm)	2.341	2.307	2.33	2.29	2.313	2.326	2.351	2.321	2.301	2.288	0.0233	0.078
	(219)	(203)	(214)	(195)	(206)	(212)	(224)	(209)	(200)	(194)		
Log time to peak (min)	1.790	1.904	1.762	1.872	1.726	1.787	1.738	1.734	1.764	1.786	0.0953	0.452
	(61.7)	(80.2)	(57.8)	(74.5)	(53.2)	(61.2)	(54.7)	(54.2)	(58.1)	(61.1)		
Log ascending slope[1]	0.125	0.003	0.171	-0.060	0.061	0.229	0.434	0.250	0.211	0.117	0.1568	<0.001
	(1.33)	(1.01)	(1.48)	(0.87)	(1.15)	(1.69)	(2.72)	(1.78)	(1.62)	(1.31)		
Log descending slope[1,2]	-0.244	-0.312	-0.206	-0.124	-0.186	-0.393	-0.375	-0.427	-0.299	-0.049	0.1095	<0.001
	(0.57)	(0.49)	(0.62)	(0.75)	(0.65)	(0.40)	(0.42)	(0.37)	(0.50)	(0.89)		
Log ratio of slopes[2]	0.354	0.292	0.393	0.072	0.264	0.624	0.808	0.680	0.495	0.196	0.2052	<0.001
	(2.26)	(1.96)	(2.47)	(1.18)	(1.84)	(4.21)	(6.43)	(4.79)	(3.13)	(1.57)		

[1] units are bpm per min [2] units should be negative but were multiplied by –1 so that a log transformation could be performed.

Table 3. Effect of various base cream preparations on average heart rate over 60 minute intervals.

	A	B	C	D	E	F	G	H	I	J	sed	χ^2
Heart rate (bpm)												
-60 – 0 min	146	147	147	143	145	127	145	135	124	132	11.1	0.283
0 – 60 min	139	140	129	144	138	123	142	120	123	128	10.7	0.141
60 – 120 min	125	132	124	137	134	122	139	120	120	132	10.9	0.587
120 - 180 min	128	126	126	131	135	119	130	125	119	124	6.7	<0.001
180 – 240 min	125	121	132	134	129	121	132	122	114	122	8.7	0.358
240 – 300 min	137	122	130	132	120	112	139	130	121	122	9.0	0.040
300 – 360 min	131	120	132	127	116	110	134	126	110	125	6.0	<0.001
Difference from baseline (bpm)												
0 – 60 min	-4.4	-5.1	-16.5	1.4	-6.6	-6.3	-2.7	-12.5	-0.9	-5.8	6.16	0.162
60 – 120 min	-16.7	-15.3	-20.1	-3.8	-10.3	-7.0	-6.5	-13.7	-5.5	-4.4	11.44	0.708
120 - 180 min	-15.0	-16.0	-21.1	-9.9	-9.8	-9.8	-15.8	-8.3	-4.1	-8.6	12.62	0.971

Discussion and conclusion

The data suggests that transdermal application of atropine will increase heart rate in the pig with the peak occurring approximately 60 minutes after application. The data also suggests that the base creams alone do not increase heart rate and that the affects of the preparations are due to the atropine itself.

Table 3 demonstrates that formulation G, which contains the complex between atropine and tocopheryl phosphates as per the invention, is consistently more effective than a similar concentration of atropine in compositions containing the lauryliminodipropionate-tocopheryl phosphates.

The evaluation of the data in Table 2 shows that there is a consistent increased efficacy of formulation G versus formulation H for log peak rate, log time to peak and, importantly, log ascending slope and log descending slope.

Further, the formulation according to the invention caused no inflammation, thus it appears possible to allow prolonged dermal contact without causing irritation.

Example 2

This example investigated the effect of transdermal delivery to pigs of morphine. The skin of pigs has similar properties to human skin and as such the pig is an excellent model for studying dermal delivery of drugs.

This study was designed to assess the level of analgesia as measured by a delay in the tail flinch response to a heat (62°C) placed on the rump following the transdermal delivery to pigs of morphine.

Flinch test data were analysed by REML (Residual maximum likelihood) with treatment and time as the fixed model and pig, replicate and flinch time at time zero as the random model. Data were initially analysed raw but because there were some skewed data at 6 h they were also log-transformed for analyses. Either analyses provided essentially the same interpretation.

The following formulations were tested:

Code	Composition
AGM	Morphine in formulation G as per Example 1.
AG	Formulation G with no morphine
AHM	Morphine in formulation H as per Example 1.
AH	Formulation H with no morphine.

Overall, the flinch time for pigs treated with preparation AGM had a greater flinch time than any of the other treatments (2.63, 2.88, 4.82 and 3.17 seconds for treatments AG, AH, AGM and AHM, Table 4). Interestingly, the response was greatest at 6 h after treatment (Figure 5) suggesting a sustained effect, particularly when compared to the control AG. In this context the flinch test was 133% greater at 6 h in pigs treated with AGM compared to AG. There was an indication that AHM had a greater flinch time at 2 h after treatment when compared to the control AH, but this was not sustained. AHM did not provide the sustained results which were obtained with AGM.

In conclusion, the data demonstrates that transdermal delivery of morphine in a formulation according to the invention (AGM) provides rapid and sustained analgesia as measured by a delay in the tail flinch response to a heat treatment at 1 to 6 h. Further, the formulation according to the invention caused no inflammation, thus it appears possible to allow prolonged dermal contact without causing irritation.

Table 4. Effect of treatment and time flinch response after heat probe application (seconds)[1]

	Time after treatment (h)					Significance (χ^2)		
	1	2	4	6	sed[1]	Treat	Time	Tr x Ti
AG	1.83	2.69	3.26	2.75	1.087	<0.001	0.062	0.45
AH	2.10	2.34	3.60	3.50				
AGM	3.96	3.40	5.49	6.42				
AHM	2.85	3.87	2.97	3.00				
AG	(0.260)	(0.411)	(0.461)	(0.413)	0.0858	0.003	0.011	0.85
AH	(0.313)	(0.335)	(0.465)	(0.438)				
AGM	(0.460)	(0.470)	(0.570)	(0.622)				
AHM	(0.410)	(0.466)	(0.440)	(0.458)				

[1]Values in parentheses are log transformed.

[2]standard error of the difference for time x treatment. For treatment and time effects multiply by 0.511 and 0.497, respectively.

Example 3

In this experiment, the efficacy of a morphine composition according to the invention was compared with the efficacy of morphine sulphate, the currently used enteral formulation of morphine. The effect was measured by comparison of times taken for a rat to withdraw its paw in response to heat when medicated and unmedicated with morphine.

<u>Materials</u>

Animals: Nine conscious Sprague-Dawley rats .weighing between 350-450 grams each

Treatment groups:

1. Control: water,
2. morphine sulphate,
3. Morphine with TPm: morphine HCl (14%) in a carrier containing water (59%) and a tocopheryl phosphate mixture (27%) (***TPm***). The TPm contained mono-tocopheryl phosphate and di-tocopheryl phosphate.

Formulations 2 and 3 were diluted with water and the final morphine concentration was made up to 5 mg/ml. For example, 0.357 grams of formulation 3 was mixed with 0.643 grams of

water to obtain a final morphine concentration of 5%. This liquid formulation was then delivered to the animals by oral gavage (tube into stomach).

Method

The experiment used nine rats that were divided into three groups. After the first treatment, the rats were rested and each group was given a different treatment. The process was repeated once more until each rat had been given each of the three treatments.

Water, morphine sulphate and morphine with TPm were given by oral gavage at a concentration of 5mg/kg of body weight. Analgesic testing was performed at 1, 2, 4 and 6 hours and at each time point withdrawal latency was measured three times on each rat (with at least five minutes rest if using the same paw).

A plantar analgesiometer designed for rapid and efficient screening of analgesia levels in small laboratory animals was used. The device applied a heat source (~45°C from an infrared light) to the animal's hindpaw and the time taken to withdraw the paw from the heat source was measured (paw withdrawal latency). The heat source (plate) provided a constant surface temperature. It had a built-in digital thermometer with an accuracy of 0.1°C and a timer with an accuracy of 0.1 second. The animal was placed on a hot plate, confined by a clear acrylic cage which surrounds the plate and paw withdrawal response was monitored. An increased time period before paw withdrawal response indicating analgesia. Each animal was tested 3 times at each time point. (ie a single rat had the heat applied to its back foot three times at each time point).

The results are illustrated in Figure 1. Both the morphine sulphate and morphine in the tocopheryl phosphate carrier caused an increase in latency indicating analgesia. The morphine in the tocopheryl phosphate carrier caused a greater latency which was maintained for a longer period of time than the morphine sulphate. That is, the morphine formulated in a carrier according to the invention provided a sustained analgesic effect for up to 2 hours following oral administration whereas the morphine sulphate only provided an analgesic effect for the first hour The standard error bars on the graph points do not overlap except at the one hour time point where the aqueous morphine sulphate and the morphine TPm formulation were similarly active. For the later time points, the morphine TPm formulation gave sustained analgesia.

Statistical Analysis: Comparison between morphine sulphate and Morphine TPm formulations.

- at 60mins $t=2.598$ ($p<0.02$)
- at 120mins $t=4.815$ ($p<0.0005$)
- at 240mins $t=4.351$ ($p<0.001$)

- at 360 mins t=3.094 (p=0.005)

Conclusion

The use of the TPm carrier provided a sustained analgesia over a longer period of time using the same amount of morphine as the morphine sulphate formulation. Whilst the results were not significant at the one hour time point, the TPm formulation was statistically significant at all later timepoints.

Vital Health Sciences Pty Ltd **3 August 2004**

Figure 1

Effect of various atropine formulations on heart rate in pigs. Data are cumulative averages over 10 minute periods and have been corrected for basal (average of 1 h before application) using covariate analyses.

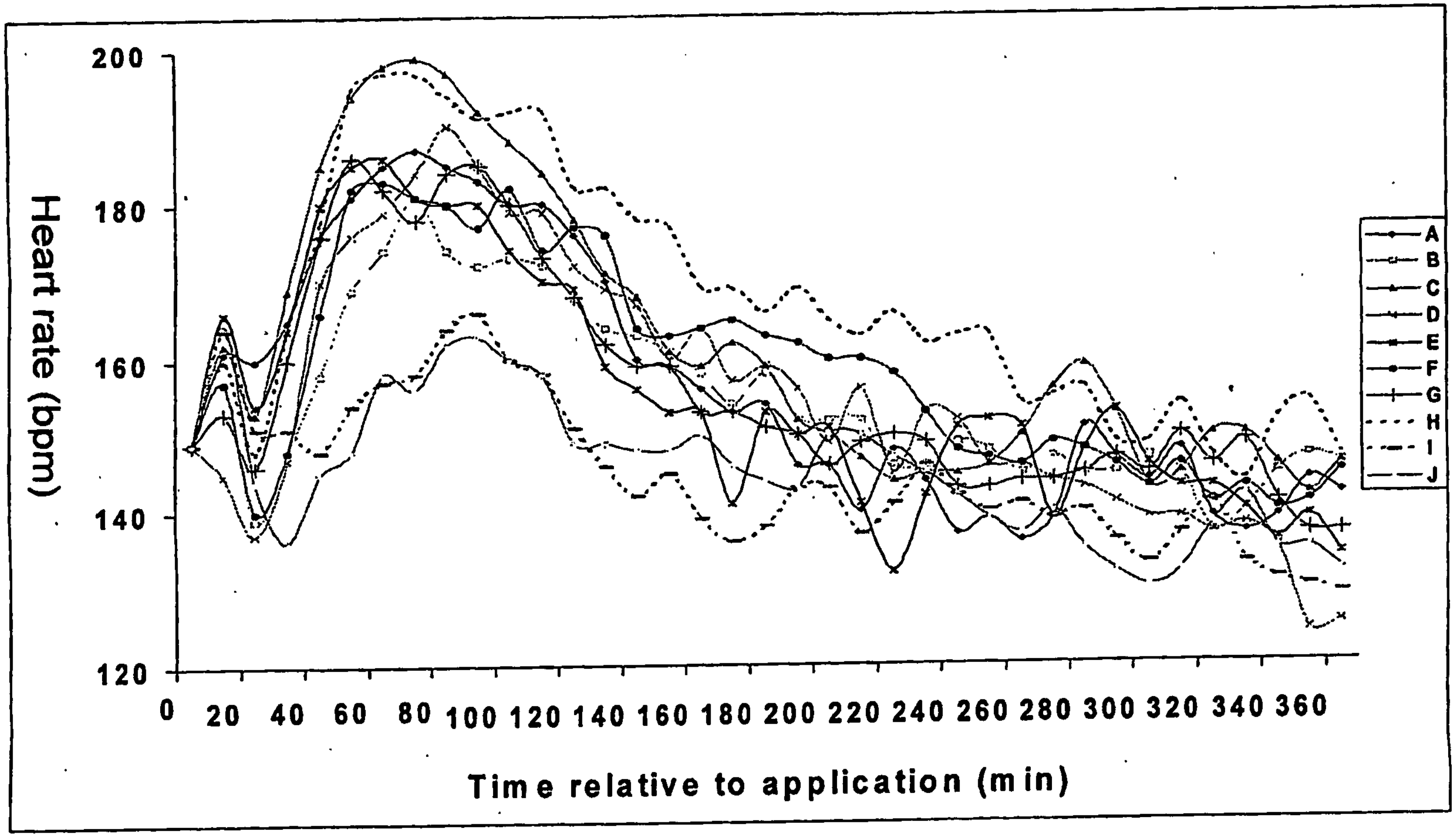


Figure 2

Typical differential of heart rate versus time curve. Data are from pig 1 during replicate 1 who was treated with preparation C (ie the very first pig used). The treatment application commenced at 0 minutes and continued for 6 minutes. The period over which differentials were averaged is indicated in red.

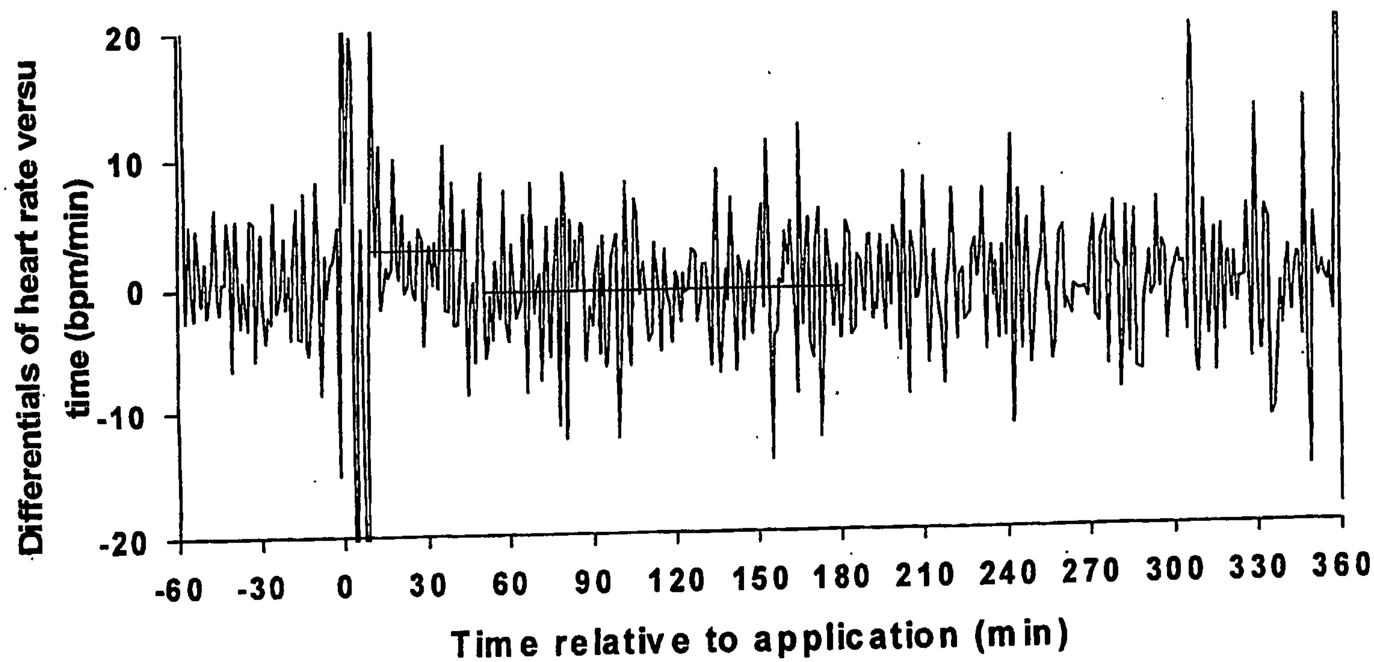


Figure 3

Effect of various base creams on heart rate in pigs. Data are cumulative averages over 10 minutes periods and have been corrected for basal (average of 1 h before application) using covariate analyses.

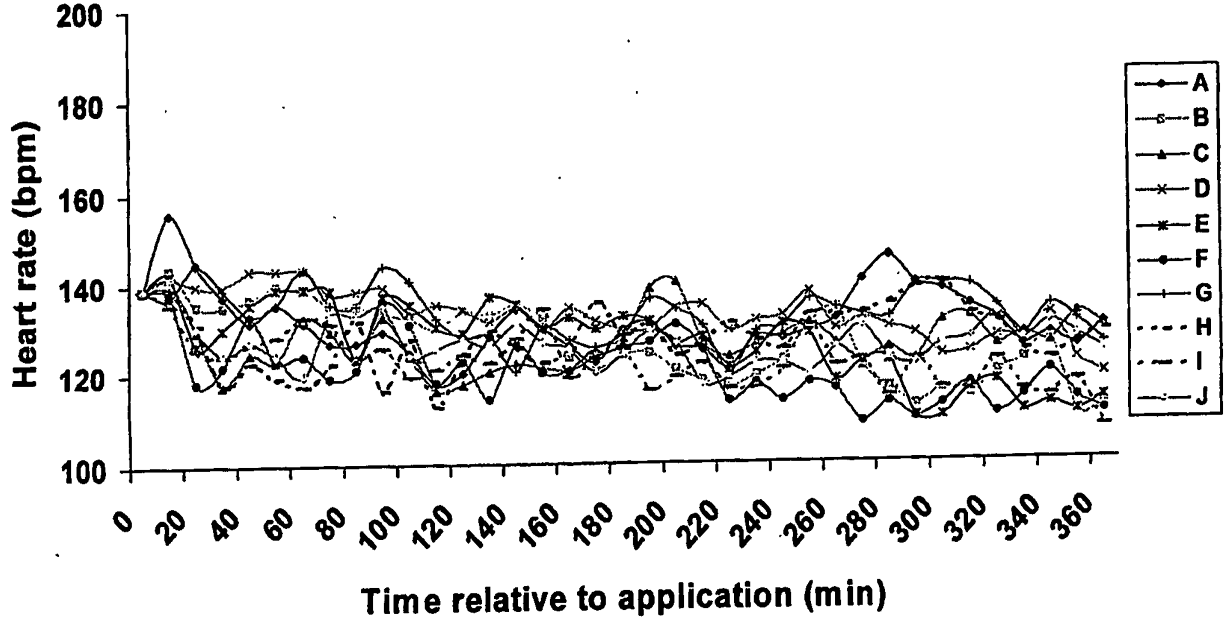


Figure 4

Typical heart rate versus time curve. Data are from pig 1 during replicate 1 who was treated with preparation C (ie the very pig used). The treatment application commenced at 0 minutes and continued for 6 minutes. The period over which differentials were averaged is indicated in red.

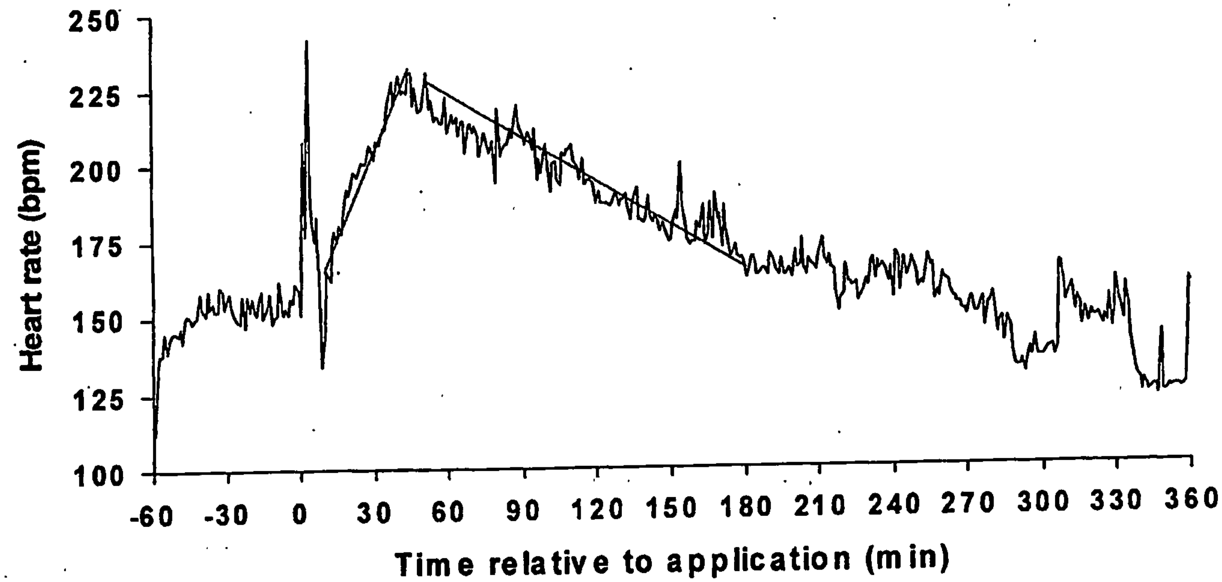


Figure 5

Effect of treatment and time flinch response after heat probe application

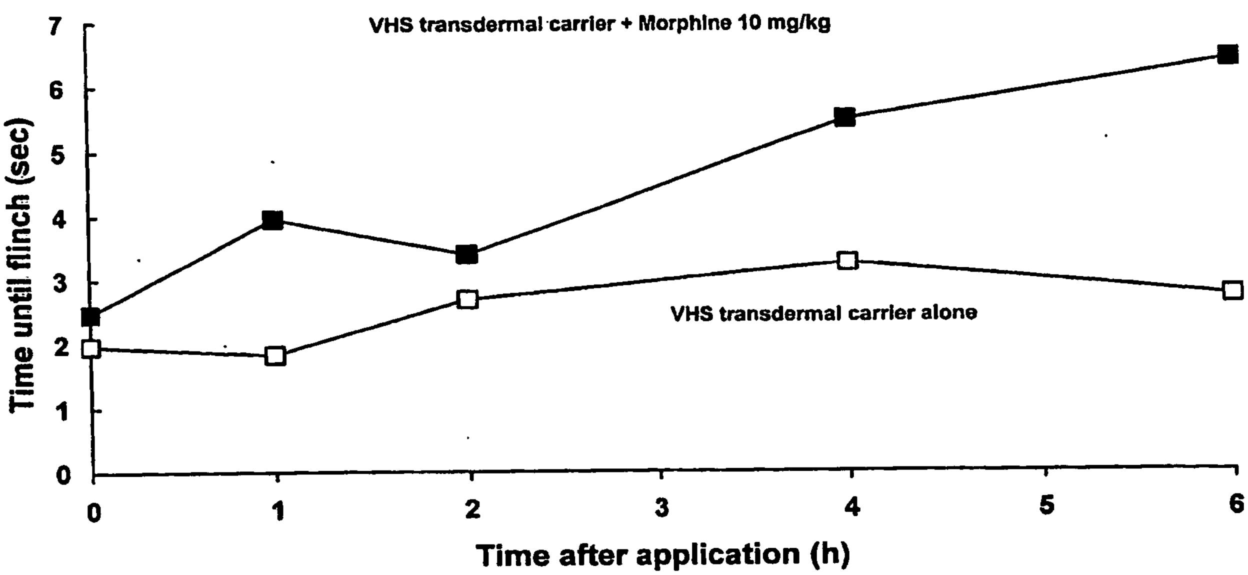
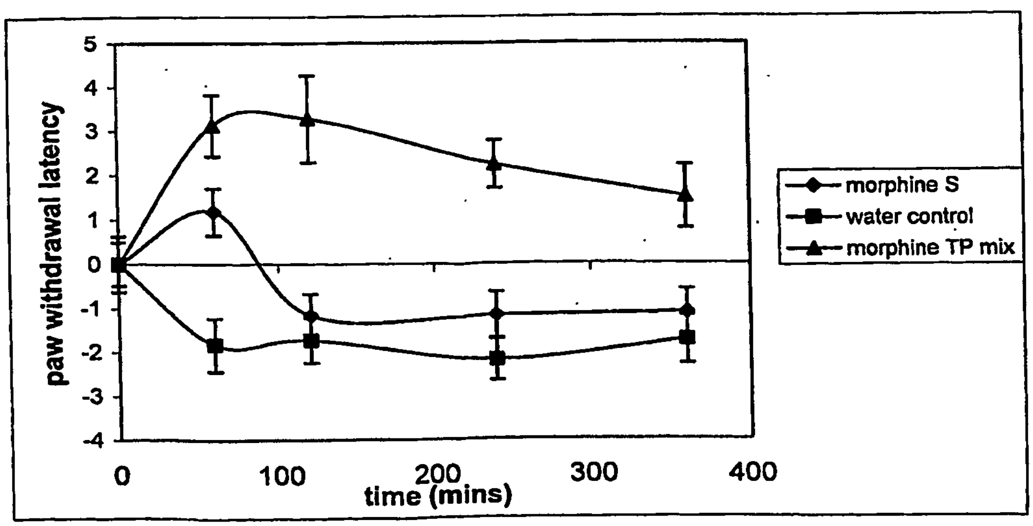


Figure 6

Effect of morphine sulphate 5 mg/kg, morphine with TPm 5 mg/kg and control on paw withdrawal latency in rats, tested over 3 hours (pooled data).



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